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ASSAYS IN THE CONTROL OF INDUSTRIAL WASTE
DISPOSAL

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BIOLOGICAL OBSERVATIONS AND TOXICITY BIOASSAYS
IN THE CONTROL OF INDUSTRIAL WASTE DISPOSAL*

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Introduction

In order that the disposal of industrial wastes into surface waters may be properly controlled, the pollutional nature of these wastes and their influence on aquatic life must be considered. The value of biological indices and measures of water pollution has been pointed out and discussed repeatedly in the past. Very recently Van Horn (19), Patrick (16) and Henderson (12) have published interesting data and discussions of various phases of this subject, with especial reference to industrial pollution. Biological indices of pollution with industrial wastes and domestic sewage (which has effects similar to those of some organic industrial wastes) have been considered in a general way also by Bartsch (2), Butcher (4), Brinley (3), Lackey (14) (15), Campbell (5), Ellis (8), Claassen (6) and Whipple (20). Ellis, Westfall and Ellis (9), Hart, Doudoroff and Greenbank (10) and Anderson (1) have recently described bioassay methods for the evaluation of the toxicity of industrial wastes to aquatic animals, as a helpful index of their pollutional potency. Important contributions of other workers have been cited by the authors mentioned above.

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Many established facts and valid conclusions pertaining to biological criteria by which the intensity of pollution can be judged already have been fully and clearly presented and need not be restated here. However, some strikingly contradictory views are frequently encountered. Therefore, the present general discussion, based on a critical survey of pertinent literature and personal experience in the field of pollution control, may be helpful in the planning of biological studies. Bacteriological investigations and methods are not considered here.

Objectives of Biological Studies

The practical objectives of biological studies of water pollution first must be carefully considered. It seems that some authors have failed to recognize, or have lost sight of these objectives. This may have resulted in some misunderstanding and incomplete appreciation of the aquatic biologist's part in the solution of waste disposal problems.

Much has been written about so-called index organisms, or biological indicators of pollution, as aids to the rapid detection of organic pollution and estimation of its intensity, or of the degree of natural purification of streams. The usefulness of some organisms, or associations of organisms, as indicators of stream conditions, in connection with pollution surveys and the classification of waters, should not be overlooked or minimized. However, the mere detection of pollution and the determination of its source, nature and extent are only steps toward broader, more worth-while objectives. It is noteworthy, also, that contamination of water with industrial wastes can be detected and measured by means other than biological ones. Sanitary and industrial engineers and chemists can well point out that physical and chemical methods, with

which they are more familiar, usually are adequate for this purpose. Granting that certain species of plankton organisms, for example, normally are abundant in polluted waters or in clean waters only, is it essential, one may ask, to identify the plankton forms present in order to evaluate the purity or quality of a given water? Are these indicators more reliable than other criteria employed in testing their reliability?

Biologists have replied that their observations reveal the actual effects of pollution on the aquatic biota, while physical and chemical tests reveal only the primary causes of these effects. This is quite true. But just what is the practical significance of the effects noted? Is an observation that twelve species of algae have disappeared from a section of stream, while five other species have become more abundant, because of pollution, of interest to anyone except a biologist? In what practical sense is the condition of the affected stream less satisfactory than it was before? It has been said that some natural biotic balance, or dynamic equilibrium, seems to have been disturbed. However, a permanent equilibrium can exist only in a stable environment, and even unpolluted stream environments and populations are notoriously unstable. Aquatic life, in general, is more adaptable to environmental changes than are individual species. Even the activities of organisms found in some streams heavily polluted with organic wastes apparently can be very nicely balanced. At least, they perform the function of waste assimilation more effectively than it can be performed by the more varied flora and fauna of a previously unpolluted stream. How, then, is the biota of the polluted stream likely to be impaired, and what is the most appropriate measure of this deterioration?

To a fishery biologist, who is accustomed to thinking of the great recreational and commercial importance of aquatic biological resources,

the answer seems obvious. The relative value of aquatic populations can be expressed in terms of the actual or potential yield of desirable aquatic species and the over-all value of this crop. The layman may overlook the fact that an abundant supply of natural fish-foods is essential for good fish production and must be protected from harm by pollution. Not only fishery biologists, however, judge pollution damage by its effect on the fisherman's catch.

Most students of the subject agree that there must be demonstrable detriment to some normal, legitimate use of a stream, or at least convincing evidence that some such use (actual or potential) is endangered, or would be dangerous to man, before the quality of the stream can be reasonably deemed impaired. Probably no such effect of pollution with industrial wastes is of more frequent occurrence than is the destruction of fish and their aquatic food and of the recreational appeal of affected waters. Also, none is more likely to attract public attention and to arouse concerted action by groups of organized sportsmen and commercial fishermen. It has been estimated (18) that at least twelve million people took advantage of the recreational fishing resources of the United States in one war-time year (1942-1943). The number of anglers has increased rapidly in recent years, and their annual catch in fresh waters is believed to amount to hundreds of millions of pounds of fish. It has been estimated also that in the year 1949 some 28-1/2 million licensed fishermen and hunters spent about four billion dollars in the pursuit of their outdoor sport. The value of fishery resources and the damaging influence that disregard of this value can have upon an industry's public relations are being increasingly recognized. It is not surprising, therefore, that some widely

accepted chemical criteria of harmful industrial pollution (such as minimum dissolved oxygen concentrations in waters receiving organic wastes) are based chiefly on the apparent water-quality requirements of fish life.

Unfortunately, the somewhat ambiguous wording of much pollution-control legislation does not help to clarify matters. Some legislation specifically prohibits pollution which is directly or indirectly inimical to fish life (6). Often, however, the discharge of any substance which is harmful to fish and other aquatic life is prohibited by law or regulation. Presumably, aquatic life in general is mentioned in order to protect all organisms of some demonstrable value to man. Waste disposal which has no effect other than the elimination of some aquatic species of no commercial value, or importance as food for fish, shellfish and game, probably was never given serious consideration in the formulation of these restrictions. It is doubtful that any industrial concern accused of causing or contributing to no more serious "damage" ever has been, or soon will be, convicted of violating such a general prohibition. This is more significant than the exact wording of the legislation involved.

Yet, aquatic biologists not infrequently have discussed biological indices of pollution and proposed measures of pollution intensity without attempting clearly to relate them to the productive capacity of waters. Fishes sometimes have not been mentioned at all, or have been dealt with merely as constituents of biotic communities, of no special interest. This may be justifiable from an academic point of view, but such a viewpoint is not shared by many practical workers in the waste disposal field, whose common goal is the prevention and abatement of harmful pollution. These workers realize that progress can be made only with the support and

cooperation of many laymen, who have little interest in the mere restoration of primitive stream conditions and of hypothetical biotic equilibria. These laymen often must be convinced that a given instance of pollution is actually objectionable, and shown just what economic or recreational benefits can be achieved through proposed, frequently costly, remedial measures.

What, then, should be the major, immediate objectives of biological studies directed toward the abatement of water pollution with industrial wastes? In the writer's opinion, the outstanding objective is the detection, reliable estimation, and explanation of the damage, or danger, to aquatic biological resources of commercial or recreational value, due to pollution. Harmful effects of industrial pollution other than damage to fisheries can be related to biological phenomena. For example, by supplying certain nutrients, such pollution of water supply sources and recreational waters sometimes can promote excessive multiplication of certain organisms which cause odors, tastes, filtration difficulties and other nuisances. Toxic wastes, on the other hand, can interfere with the natural purification of waters receiving sewage and other organic wastes, by inhibiting the development of a typical pollutional flora and fauna. These undesirable results of pollution usually are obvious or readily detectable by standard tests used in the routine control of water quality. Special biological studies may be necessary, however, in order that indirect damage of this nature may be correctly explained and attributed to its true, primary causes, or foreseen and avoided. These somewhat involved studies are indicated whenever local conditions and water uses are such that serious interference with the use of contaminated waters for purposes other than fishing is already apparent or likely. In this paper, only investigations having to do with damage

to fisheries, which can be detected and evaluated only through appropriate biological observations, are considered fully. It is believed that such studies should constitute a part of every comprehensive investigation of industrial pollution of surface waters.

The Need for Biological Studies

Investigators ostensibly seeking to confirm or refute reports of damage to aquatic life by pollution not infrequently proceed into the field with no equipment other than that required for the collection and chemical analysis of water samples. The investigations are often deemed complete when not a single biological observation has been made and recorded at the streamside. Likewise, when a sample of an industrial effluent is brought into a laboratory for examination and estimation of its possible lethality to aquatic life, an indirect approach to its biological properties is generally favored. The biochemical oxygen demand test is the only bioassay that has come into general use. When the B.O.D. of a complex waste is found to be great, the possibility that toxicity to aquatic animals may be an even more important property is often overlooked entirely. In any event, highly toxic components which are not known to be present, or cannot be readily separated and measured chemically, frequently are not detected by the chemical tests which are undertaken, and so receive no attention.

The chemical nature of a waste or contaminated water having been determined, some of the available literature on the toxicology and water-quality requirements of aquatic life is consulted. Original sources of such information, which reveal the complexity of the problems investigated and the deficiencies of the data reported, are seldom studied and appraised

critically. Concise summaries and compilations of miscellaneous lethality data, presented with a minimum of qualifying detail, are far more popular references. Indeed, there is a constant demand for even simpler summaries which would facilitate indiscriminate misapplication of experimental data. A table showing arbitrarily what concentration of every known component of polluted waters may always be deemed barely safe for fish life probably would be regarded by many as an ideal summary. Such simplicity can be achieved only by disregarding or slighting the influence of many complicating factors of great import (7) (8). It is well known, for example, that ammonium salts can be far more injurious to fish when dissolved in hard, alkaline waters than in soft, acidic waters. Salts of copper and many other substances, on the other hand, are most harmful in waters of the latter type. The toxicity of a complex mixture of chemicals cannot be definitely predicted even when the concentration and toxicity of each of the separate components have been reliably evaluated. Because of synergy or antagonism, the mixture can be much more harmful, or less harmful, than any of the important constituents alone.

Usually it is not difficult, of course, to determine by observation or experiment how aquatic organisms are actually affected by any given pollutant or combination of pollutants. Studies in the field reveal the over-all effect on organisms in their natural habitat. Toxicity tests or bioassays, performed under controlled conditions in the laboratory, show how toxic the pollutants actually are, to the selected living organisms used as reagents, in any given natural medium. The appearance and behavior of affected animals often suggest the cause of observed injury. For example, when fish were found to be dying recently in tap water in laboratory

aquaria, their appearance indicated the lethal action of copper from some unknown source. A simple chemical test for this metal only was necessary, therefore, for establishing conclusively the cause of the mortality. Chemical tests contribute to an understanding of the causes of biological effects of pollution, and the results suggest remedial measures. It is not often necessary or justifiable, however, to deduce the biological effects of pollution from chemical data, when direct observation is possible.

Unfortunately, a few biologists have encouraged a different view, perhaps quite unintentionally. Some of their well-known writings tend to produce the impression that the detection and measurement of stream pollution harmful to fish life is almost entirely a matter of physical and chemical examination of waters. Aquatic biologists, for whom the instructions may have been chiefly intended, should need no indoctrination concerning the value of biological observations. Other readers may have concluded, however, that the only important function of the aquatic biologist is to show what pollutional conditions should be ascertained by physical and chemical means, and how the data should be interpreted.

Generally speaking, when some effect of a demonstrable alteration of an aquatic environment cannot be observed or measured, for any reason, one may be justified in estimating it inferentially. Such a procedure is unsound, however, when the effect itself can be readily determined through direct observation. It should not be necessary to point out the need for biological studies and tests, which show what pollution actually does to the aquatic biota, when possible damage to aquatic life is under investigation. The results of a physical and chemical study can be no more reliable than is the investigator's judgment concerning the biological significance of the

data. Yet, the number of aquatic biologists who, in the past, have found employment and an opportunity to specialize in the practical field of pollution study and abatement has been very small. It testifies to the existence of an anomalous and deplorable state of affairs in this branch of applied science. Fortunately, there is now some evidence of increasing recognition of the value of the aquatic biologist's services in connection with waste disposal and pollution control programs. Other workers in the field are now receiving some special biological training.

Not only these other workers, but also biologists undertaking studies in this practical field, should remember that the best approach to the solution of any problem usually is the most direct approach. Their investigations should be planned accordingly, and unnecessary steps of no evident practical value should be avoided. When the hazard to human health associated with bacterial contamination of water supplies, due to pollution with domestic sewage, is to be estimated, certain bacteriological tests are most appropriate. Other, supplemental, biological indices of the sanitary quality of the water are of questionable value in this connection. The nature of the bacterial flora is deemed a more reliable and specific index of this kind of pollution and hazard than is, for example, the plankton population. The actual effects of pollution on fish life often can be studied, and such studies should need no justification.

Nature of Biological Studies

How can the dominant objectives indicated earlier in this paper be achieved best? What particular observations yield the most informative and useful data? These questions are frequently asked by administrators and investigators who are undertaking general biological studies of water

pollution, or are dissatisfied with past results. Opinions on this subject vary. Nevertheless, for those to whom the following suggestions appear to be reasonable, they may be a helpful guide in the planning of a biological study program.

As a rule, the personnel and facilities available for these practical studies are necessarily limited. Although the collaboration of a number of specialists in different branches of aquatic biology may be desirable, it is rarely feasible. For this reason, the suggestion that nearly all species of aquatic animals and plants be always distinguished, identified and considered (16) is not often acceptable. Probably no biologist has the necessary broad taxonomic training and also adequate knowledge of ecology, physiology, water chemistry and other pertinent subjects. Furthermore, one may reasonably question the ability of biologists to interpret much of the information so obtained in terms of the productivity and the economic and recreational value of the waters studied.

Some general relations between fish populations and the nature and abundance of plants and invertebrate animals found in typical clean and polluted waters have been noted. There is no evidence, however, that the variety of other organisms present is always a reliable index of fish production. The entire aquatic economy is still imperfectly understood. For this reason, quantitative studies of phytoplankton and the fertility of waters also are not often very instructive. Aquatic plant life is the chief basic source of the organic food of animal life in natural waters. Artificial fertilization of fish ponds is now widely practiced. Such fertilization may tend to restrict the variety of plant plankton, but stimulates the multiplication of some species and is deemed favorable for fish

production. However, the excessive fertilization of some contaminated waters apparently has been detrimental to desirable fish species (11). The ideal degree of fertility cannot be stated and must vary greatly, depending on other features of the environment. Research directed toward a better understanding of the interrelations of all plant and animal species of the aquatic community and their responses to pollution is highly desirable and should be encouraged. However, there are more direct and satisfactory procedures now available for evaluating fish habitats than those mentioned above.

1. Comparative studies of fish populations and fisheries

It is futile to speculate whether a given section of stream is or is not fit for desirable fish species at the time of a pollution study if the presence of an abundant, varied and healthy population of such fishes is readily demonstrable. If fishermen flock to this section of stream because they are successful there, no evidence of unnatural chemical conditions or an abnormal food-chain will convince them that they should go elsewhere for their sport. Conversely, apparently tolerable water-quality conditions and an abundance of natural fish-foods are of no value when the food supply is not utilized by desirable fish species, because these are repelled by some pollutant or cannot propagate successfully. Even when fish are abundant, they are of no value if they are not sought and utilized, because of their seriously impaired flavor or heavy infestation with parasites, which may sometimes result from pollution. Comparative studies of fish and fishing in streams above and below industrial waste outfalls, and also in other similar but unpolluted waters nearby, usually are more instructive, therefore, than any other pertinent observations.

A creel census is the best method of evaluating fishing success where fishermen are sufficiently numerous. The fishing intensity, the kinds of fish taken and the average take per man-hour of fishing effort thus can be reliably determined. Fishermen can be found on the fishing grounds or at popular boat landings. Much valuable, specific information can be obtained by questioning both anglers and commercial fishermen, but their general reports and opinions should be carefully weighed. Fishermen have been known to decry damage to fishing by pollution, yet to continue fishing in the polluted waters when similar, uncontaminated waters were as easily accessible.

Fish populations also can be sampled by means of nets and other devices, such as the "electric seine". Experimental gill-nets, with meshes of varying size, and traps such as fyke-nets are useful in some waters, especially for taking the larger fishes. However, seines are most generally useful in streams for capturing fish of different sizes and collecting a large number of samples from different fish habitats in a relatively short time.

Unfortunately, only gross estimates of the relative abundance of fishes usually can be derived from the results of such sampling, especially in large streams. When compared with available methods for quantitative sampling of plankton and bottom life, methods for the sampling and estimation of fish populations of streams appear to be least satisfactory. Still, it is doubtful that quantitative estimates of available fish-food, even in unpolluted streams, are often more reliable indices of relative fish production than are data obtained through fish population studies.

The mobility, or migratory tendency, of many fishes is a complicating factor which must be considered. In the course of studies of polluted waters

in Louisiana, very rapid repopulation of damaged streams with fish migrating or returning from contiguous waters was observed. In one such stream not a single fish could have remained alive in about a hundred miles of the stream channel, because of the discharge, in early winter, of an oxygen-depleting and toxic pollutant. Within a few weeks after the pollutant had been flushed out with fresh water, a variety of fishes could be taken in large numbers with nets at various points. Commercial fishing for migrating buffalofish and other species was reported to be unusually good. Angling during the following year did not appear to be very seriously impaired; and the variety and number of fish killed when the pollution recurred after a year also revealed no pronounced, lasting effect on the fish population. Repopulation of many streams elsewhere cannot be nearly so rapid, however. The absence of some migratory fish species in a section of stream may be due not to local conditions, but to a pollutional or other barrier to migration elsewhere. On the other hand, a stream section may contain temporarily many more migratory fishes than it can permanently support. These are important considerations when comparative studies of the fish populations of different waters are undertaken.

The kinds of fish present must be distinguished, and their habits, size, condition and relative value must be noted. An abundance of very young fish and of healthy adults of non-migratory forms is indicative of successful reproduction and survival of fish in the area studied. It is much more instructive than the number of transients which may be taken by fishermen. Rates of growth, determined through examination of scales, or otherwise, also can be instructive. Small forage fishes are important as food for the larger predaceous fishes. However, a pronounced increase of the numbers of

rough fish, such as carp and bullheads, which often occurs in polluted waters, does not compensate for a great reduction of the numbers of game and food fishes which are deemed far more valuable. Tainting of the flesh of fishes in waters contaminated with phenolic and other pollutants has been reported to make it unfit for human consumption. It should be obvious that some of the most important effects of pollution often can be overlooked entirely by investigators who neglect the study of fish populations.

2. Comparative studies of bottom-living organisms (benthos).

When there is convincing evidence that the fish population and fishing in a stream have not been impaired by pollution, an intensive study of fish-food organisms may be superfluous. When such damage is demonstrable, on the other hand, its cause should be determined. The destruction of the food supply of valuable fish species is one of the possible factors of major importance. Some fish-food organisms are more sensitive to organic or toxic wastes, or to changes of the character of the stream bottom, than most fishes; and their disappearance can have serious consequences. However, other direct and indirect damage to fish life should not be overlooked. The multiplicity of ways in which pollution can be harmful, and sometimes also favorable, for fish life has been well pointed out by Hubbs (13). All possibilities should be considered and explored.

Aquatic organisms are not all equally desirable and available as fish foods, and fishes have varying feeding habits. The feeding habits of important species in an area under study are revealed by their stomach contents. Studies have shown that bottom-living organisms are important as foods of many of the most valuable fresh-water fishes, and that some are utilized

far more than others. For example, in some trout lakes studied by the author, midge larvae and pupae were numerous both in bottom samples and in trout stomachs. Small clams and oligochaete worms were numerous in the bottom samples, but apparently were of negligible importance as food of the trout. Large aquatic insects, on the other hand, were not common in bottom samples, but were frequently eaten and made up more than half, by weight, of the stomach contents of trout examined. The total weight of potential food found per acre of habitat is not, therefore, very instructive. The relative importance of the different organisms as fish foods must be estimated. However, identification of all species is not deemed essential. Only groups of related species with similar habits must be distinguished from other forms characterized by very different habits. Every well-trained fishery biologist and limnologist should be able to recognize these forms.

Quantitative bottom samples can be taken with any of a number of standard devices, such as the Petersen dredge, the Ekman dredge and the Surber square-foot sampler. The choice of sampler depends on the nature of the bottom. The marginal fauna and attached aquatic vegetation, with the animals living on it, also should be sampled.

The nature and quantity of fish-foods present above and at various points below a source of pollution can explain the disappearance or scarcity of desirable fishes in the polluted water. Also, they can be useful indices of the suitability and productivity of fish habitats when conditions are such that only a very limited amount of reliable information about the fishes present can be obtained by direct observation. When it can be shown that all important food organisms have been destroyed, or their numbers have been greatly reduced by pollution, one may reasonably conclude that fish

production is impaired, even in the absence of direct evidence. Thus, damage to fisheries sometimes can be demonstrated most easily and quickly, and even most convincingly (e.g., where fishes are predominantly migratory, or very difficult to collect), through comparative studies of the invertebrate fauna. However, even where the influence of pollution on the bottom fauna is very striking, serious indirect damage to the fish population may not always be assumed. As an example, the results of a preliminary biological survey of a river receiving the effluent from a pulp and paper mill can be cited.

A previous investigation by a group of engineers and chemists failed to reveal pollutional conditions which could be assumed to be detrimental to the normal aquatic life. Dissolved oxygen, for example, appeared to be quite adequate (well above 5 p.p.m.) at all sampling points, although the stream flow at that time was not far in excess of the minimum flow. Nevertheless, it was found that the bottom fauna changed abruptly at the source of pollution. Above this point, burrowing mayfly nymphs were numerous, unionid clams were very common, and other clean-water organisms were present in the bottom samples. Below the source of pollution (1 to 10 miles downstream) these fish-food organisms were not found, but oligochaete worms, small chironomid (midge) larvae, and other tolerant forms were much more numerous than they were in the unpolluted stream section above. Small forage fishes (cyprinids) also were plentiful here. Thus, there was no evidence of conditions lethal to fish life, nor of lack of food both for small fishes and for large predaceous game fishes, such as black bass, within this markedly polluted zone. Farther downstream, however, 30 to 43 miles below the source of pollution, the tolerant, pollutional bottom organisms were again

comparatively scarce, while almost no recovery of the clean-water bottom fauna was apparent. Plankton was not found to be important anywhere in this rapidly flowing river.

Peculiarities of the bottom fauna and flora can be indicative not only of indirect damage to fish life by pollution, but also of the nature and source of pollution which is known to be directly harmful to fishes. The causes of the disappearance or occasional mortality of fishes in polluted streams, and especially in streams subject to intermittent pollution, are not always easily determined. The effects of pollutants on the distribution of bottom organisms with long life-cycles are often more lasting, more distinctive and more readily detectable than the effects on the distribution of fishes. They can be very helpful clues. Indicator organisms which are often dominant in waters polluted with organic wastes have been intensively studied. Forms which are outstandingly susceptible to harm by different kinds of pollution have received less attention. These may be termed "negative indicators", inasmuch as their presence indicates the absence of a particular kind of harmful pollution. Mayfly and stonefly nymphs, for example, are known to be among the first forms to disappear in waters subject to excessive organic pollution. Some air-breathing (pulmonate) snails are not so readily affected by organic pollutants such as sewage, but were found by experiment to be far more susceptible than mayfly nymphs to some lethal agents, such as dissolved copper. The selective action of toxic pollutants, as well as of organic pollution, on bottom-living organisms should not be overlooked and deserves much additional study.

3. Comparative studies of plankton.

In many rapidly flowing streams and other waters, plankton is naturally scarce, and fish feed mostly on bottom-living organisms. In some waters, however, the bottom life is of minor importance as food for valuable fish species, which feed directly on plankton and on smaller plankton-eating fishes. Fishes, other than bottom-living forms such as catfish, were quite numerous in sections of sluggish streams and canals which were practically devoid of bottom life, because of pollution with heavy oil-field brine and depletion of dissolved oxygen in the dense water stratum near the bottom. Benthic food evidently was not essential for these varied fish populations. Furthermore, some bottom-living animals depend on the plankton, which they capture, for their food supply. Therefore, studies of the effects of pollution on the abundance of plankton sometimes are indicated. Studies of the larger zooplankton, which is eaten by many fishes, including young and even adult game fish, are especially instructive. The zooplankton thrives only where suitable plant food is sufficiently abundant. It can be readily collected or concentrated with plankton nets.

The abundance of plankton is subject to great and rapid fluctuations even in unpolluted waters. For this reason, careful, quantitative studies of plankton usually are worth-while only if they extend over a considerable period of time. Brief observations sometimes can be very misleading. The abundance of long-lived plankton-eating animals can be much more instructive.

Plankton originating in suitable breeding areas may be collected in a stream at some distance from such areas, and under conditions under which the organisms cannot thrive. At best, the nature of the plankton is indicative only of the quality of the water at the time of collection. It does not often

reveal the existence and source of harmful intermittent pollution which occurs at long intervals. For these reasons, the value of the planktonic forms as indicator organisms is limited. Nevertheless, some attention to suspended organisms in all waters under investigation is recommended. An unusual abundance of phytoplankton suggests the fertilizing action of recent, possibly serious, organic pollution of the water. The presence of pollutorial forms, such as detached fragments of sewage fungus (Sphaerotilus), can be a reliable index of organic pollution which is not readily detectable by other means. Recent contamination of a river water which was believed to be free of such pollution, by investigators who had been collecting samples for routine chemical examination, thus was clearly indicated. By watching for fragments of Sphaerotilus while moving upstream to the place of their origin, the source of pollution which had been overlooked was soon located.

4. Experimental studies (bioassays).

The need for experimental studies of the toxicity of pollutants to aquatic life already has been indicated. The toxicity bioassays should not, as a rule, be depended upon to show whether damage to the aquatic biota has or has not occurred as a result of past pollution. Much more reliable evidence usually can be obtained through field work suggested above. However, toxicity bioassays undertaken for the following purposes can be highly instructive:

- A. To determine whether observed pollution damage is attributable to acute toxicity (i.e., more or less rapid, direct lethality) of any of the pollutants involved, or must be ascribed to other harmful properties;

- B. When toxicity of an industrial plant effluent has been demonstrated, to determine which component wastes or fractions contribute to this toxicity, thus facilitating identification of the toxic substances;
- C. To estimate a safe rate of discharge of any possibly toxic waste, or mixture of wastes, which can be stored and released at a controlled rate;
- D. To estimate the possibility of damage to the aquatic life of a stream which is to receive wastes from a proposed industrial plant, when wastes from another plant using a similar process, or synthetic wastes, are available for testing;
- E. To evaluate the effectiveness of any process for the treatment of a toxic industrial waste.

It is noteworthy that extensive mortality of fishes has resulted frequently from the rapid discharge of some industrial waste, the toxicity of which had not been realized. Many of the soluble components of industrial effluents are potentially toxic, in the sense that they can be directly lethal to aquatic organisms when present in sufficiently high concentrations. However, their safe disposal by adequate dilution with available stream water often presents no difficult problem when their harmful effects are known. Temporary storage and controlled discharge of the most toxic wastes sometimes is indicated. Toxicity bioassays do not show exactly how much dilution is necessary. They do show, however, what concentrations of a waste, or of a mixture of different wastes, diluted with the receiving water, are definitely lethal to the test organisms selected. An appropriate, reasonable margin of safety then can be provided for in estimating a safe disposal rate, or the degree of waste treatment required.

Heavy fish mortality occurred repeatedly in a stream receiving wastes from a Kraft process pulp and paper mill. These wastes were being retained in a large basin, and the rate of discharge varied with the stream flow. However, only the oxygen demand of this organic waste and its observed effect on the dissolved oxygen content of the stream water at several sampling points had been considered in regulating the discharge. Toxicity of the stored waste to fish, in the presence of sufficient dissolved oxygen, was easily demonstrated through laboratory tests. The need for routine toxicity bioassays was clearly indicated. It was found that all of the waste produced could be disposed of by controlled dilution with the available stream water without exceeding a concentration which was judged to be reasonably safe. No more fish mortality was seen or reported during three years of close observation thereafter. Only a reliable criterion of the pollutional strength of the effluent, for use in regulating discharges, evidently was needed in this case.

A larger mill located on another stream had a similar problem, and a similar solution was sought and achieved temporarily. However, calculations based on the bioassay results showed that the wastes produced and impounded in the course of a year could not be adequately diluted with all of the normally available water (mean annual stream flow). The comparatively new mill in question evidently should not have been located on that particular stream, without provisions for adequate waste treatment. The properties of the waste could have been foreseen, even though effluents from industrial plants using basically the same process can differ markedly.

Facilities for the evaluation of the toxicity of industrial wastes are not very costly, and the bioassay procedures are not difficult. These

tests could, therefore, be performed in industrial laboratories and by governmental agencies charged with the control of waste disposal much more frequently than they have been in the past. Bioassays with fishes as test animals are especially useful when direct damage to fish life is under investigation. Tests with other aquatic organisms, such as those of importance as fish food, also can be made, however. Water fleas of the genus Daphnia, which are very sensitive to many toxic pollutants, have been used by a number of investigators. Their convenient, small size, short life span and rapid parthenogenetic multiplication in the laboratory, where they can be readily cultured, as well as their great sensitivity, have been regarded as advantageous characteristics. The refinement and standardization of bioassay methods designed for more or less routine application has been undertaken by a section of the Research Committee, Federation of Sewage and Industrial Wastes Associations. Some recommendations of this committee are to be published soon.

Experimental studies of the reactions of fishes to the presence of various pollutants in water have yielded some interesting results (17). For various reasons, reaction tests have not yet proved very useful, however, in the routine control of waste disposal.

Summary

Biological studies of industrial pollution of water should be directed primarily toward reliable estimation and understanding of the effects of pollutants on aquatic resources of economic and recreational import. Industrial waste disposal should be controlled so as to avoid any direct or indirect damage to these resources. These objectives often cannot be

achieved through physical and chemical examination of wastes and receiving waters, and through studies of those aquatic organisms which are of no immediate value to man, and which are considered only as indicators of stream conditions. When it is not necessary, inferential estimation of pollutional damage to fish life is discouraged, and a direct approach to the evaluation of fish habitats and their productivity, and of the toxicity of wastes, is recommended. Comparative studies of fish populations and fishing success in comparable polluted and unpolluted waters are most pertinent. Studies of the bottom fauna (benthos) and of plankton also can be very instructive, but the relative importance of these various organisms as food for desirable fish species should be a major consideration in the conduct of such studies. Toxicity bioassays of industrial wastes help to explain and to predict the effects of industrial pollution and of remedial measures.

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